Microsatellite genotyping of brown crab Cancer pagurus reveals fine scale selection and 'non-chaotic' genetic patchiness within a high gene flow system

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Title: Microsatellite genotyping of Brown crab (*Cancer pagurus*) reveals fine scale selection and "non- chaotic" genetic patchiness within a high gene flow system.

Running page head: brown crab biocomplexity

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ABSTRACT: Brown crab, *Cancer pagurus*, supports one of the most important European fisheries, however, spatial patterns of connectivity and adaptation are largely unknown and difficult to identify due to the species' life history which entails distinct dispersal characteristics during larval and adult life stages. To address this limitation spatial-temporal genetic structure was assessed, using 8 microsatellite loci, across the majority of the species' NE Atlantic distribution. Neutral genetic structuring revealed a background of high gene flow throughout the region, with a superimposed pattern of chaotic genetic patchiness (CGP) linked to stochastic recruitment variability. The CGP was geographically patterned, being prevalent among English Channel samples and absent among North Sea samples, suggesting specific biological (e.g. reproductive ecology) and environmental (seascape) drivers. Such recruitment variability may compromise stock resilience and must be considered within spatial management strategies. Another prominent feature was the pronounced differentiation of males sampled within a fjord (Gulmarsfjord) from all other samples, at a single locus exhibiting the effects of divergent selection. Gulmarsfjord females were genetically similar to all other 'non-fjord' samples, and exhibited a comparative level of differentiation at the outlier locus from the Gulmarsfjord males. Due to known dispersal differences between the sexes, the pattern within Gulmarsfjord can be explained by the intermingling of allochthonous females with resident, locally adapted, males and demonstrates the occurrence of fine-scale local adaptation in this species. Collectively, the study highlights how considerable intraspecific eco-evolutionary diversification can occur despite high levels of dispersal / gene flow.

Key words: adaptation – gene flow – dispersal – sweepstakes recruitment – conservation - sustainability

INTRODUCTION

Genetic studies have yielded many insights into marine intraspecific biodiversity with important findings including the detection of significant genetic population structuring (Shaw et al. 1999, Knutsen et al. 2011, McKeown et al. 2015) and adaptation (Hemmer-Hansen et al. 2007a, Poulsen et al. 2011, Therkildsen et al. 2013a) in systems where high gene flow would be expected to prevent such differentiation (Palumbi 1994, Waples 1998). Population genetic structure and adaptation, as components of intraspecific biocomplexity, are thought to be significant factors underpinning species / population sustainability and evolutionary potential (Iles & Sinclair 1982, Ryman et al. 1995, Ruzzante et al. 2006, Therkildsen et al. 2013b). The ongoing depletion of marine populations through fishing, and demographic changes associated with predicted future climate change, are adding to the impetus to resolve: (i) spatial / temporal patterns of neutral and adaptive genetic structure (Reiss et al. 2009); (ii) historical and contemporary drivers of structuring (Hemmer-Hansen et al. 2007b); and (iii) the significance of such diversity on ecological and evolutionary timescales (Stepien et al. 2009).

While there has been considerable research on fishes with mobile larval and non-larval stages, genetic studies of crustaceans have typically focused on taxa with sedentary adults (Jorde et al. 2015). In this context the brown crab, *Cancer pagurus* (L.), occurring continuously in shallow shelf waters of the NE Atlantic from the Lofoten Islands (Norway) to Morocco (Bennett 1995) and supporting one of the most important commercial European fisheries, represents an interesting candidate for investigation as both larval and adult stages have potential for substantial dispersal. Adults are described as benthic and mobile, but there are pronounced dispersal differences between the sexes: males being largely resident, making

short random movements within small territories, while females migrate significantly longer distances, and more frequently, than males (Edwards 1979, Bennett & Brown 1983, Latrouite & Le Foll 1989, Ungfors et al. 2007). In the English Channel female migrations of up to 200 nautical miles have been reported with some crabs achieving a mean speed of 1.07-1.62nautical miles per day (Pawson 1995). The pelagic larval stage lasts for approximately three months (Eaton et al. 2003, Weiss et al. 2009, Hunter et al. 2013) and while little is known about the ecology of juveniles they are rarely caught in offshore waters, suggesting that adult crabs only move to deeper water as they grow and reach maturity.

Tagging studies have revealed that adult female migrations are consistently against prevailing currents (Hunter et al. 2013, Ungfors et al. 2007). As the larvae are poor swimmers likely to passively drift while entrained in currents, it has been suggested that contranatent female migrations are spawning behaviours aimed at facilitating return to areas of maternal origin. Even in the absence of additional extrinsic factors, the seemingly counter active dispersal of females and larvae is expected to limit 'life-time dispersal' and may thus influence spatial patterns of recruitment and structuring of reproductive populations. Tagging, fishery landings data and sex-specific growth rates variously suggest some demographic independence between the areas of brown crab abundance in the Celtic Sea, English Channel, North Sea and Bay of Biscay (Pawson 1995). Within the North Sea, the seasonal jet-like circulation associated with the Flamborough front is predicted to prevent exchange of larvae between areas north and south of the front during spawning time (Eaton et al. 2003). In the English Channel, larval surveys have reported distinct western and eastern centres of larval abundance separated by a central area of low / no larval occurrence, and hydrodynamic modelling has indicated insufficient larval transport rates to connect these spawning areas (D. Eaton, unpublished data).

Population genetic structure of Brown crab has to date been studied only in Scandinavian waters, where Ungfors *et al*. (2009) reported no significant genetic differentiation among samples spanning 1300km of waterway distance within the Norwegian Sea, Skagerrak and Kattegat. However, genetic structuring may vary throughout a species' range and failure to identify local populations may lead to local overfishing and ultimately severe declines. While the females are highly fecund (0.5 - 2.9 million eggs per brood; Edwards 1979, Ungfors 2007), paternity analysis suggests single paternity of broods (McKeown & Shaw 2008b). Such a reproductive ecology, alongside the selective harvesting of females (Bennett 1995), which are currently regarded as overexploited, may enhance the susceptibility of brown crab to genetic erosion (McKeown & Shaw 2008b).

The objective of the present study was to test the general hypothesis of genetic panmixia in brown crab throughout a considerable portion of the species' range, with a specific focus on the English Channel and North Sea. Some genetic studies of crustaceans have reported macro-geographical homogeneity with structuring apparent only at regional scales (e.g. Domingues et al. 2010), while other studies have reported fine scale spatial / temporal genetic structuring (Selkoe et al. 2010). To encapsulate such potential complexity broad- and fine-scale spatial-temporal patterns were assessed. Furthermore, comparative analyses of males and females were performed to identify differences that may be associated with sex-specific ontogenetic movements. The sampling strategy also encompassed distinct seascape features (e.g. samples collected within semi-enclosed water bodies such as bays and fjords) to examine the effect of local hydrodynamic environments. This sampling design permitted interpretation of the mechanistic underpinnings and eco-evolutionary significance of complex patterns of genetic diversity which included evidence of broad-scale genetic connectivity, fine-scale adaptive divergence of a fjord sample, and regional variation in genetic patchiness.

MATERIALS AND METHODS

***Sample collection and molecular analyses*.** Spatial/temporal sampling of adults throughout the NE Atlantic was performed using both research (CEFAS) and commercial vessels (See Table 1 and Fig. 1 for sample information). For each sample crabs were captured using multiple baited pots within a localised area (maximum distance among pots ~200 metres) over a single day with tissue biopsies preserved in ethanol. Although adult crabs cannot be reliably aged, samples were considered to consist of multiple age cohorts. For the samples collected on board CEFAS vessels, the majority of individuals were identified as male or female, which permitted downstream separation in statistical analysis.

Total DNA was extracted using a standard CTAB-chloroform/isoamylalcohol method (Winnepenninckx *et al*. 1993). All individuals were typed at eight microsatellite loci (Cpag15, Cpag1b9, Cpag2a5-2, Cpag3a2, Cpag3d7, Cpag4, Capg5d8, Cpag6c4b) following McKeown & Shaw (2008a).

Statistical analysis. Genetic variation within samples was characterised using number of alleles (N_A), allelic richness (A_R; El Mousadik & Petit 1996), observed heterozygosity (H_O), and expected heterozygosity (H_E) (Nei 1978), all calculated using GENALEX 6.2 (Peakall & Smouse 2006). Genotype frequency conformance to Hardy-Weinberg equilibrium (HWE) expectations and genotypic linkage equilibrium between pairs of loci were tested using exact tests (10,000 batches, 5000 iterations) in GENEPOP 3.3 (Raymond & Rousset 1995). Deviations from HWE were measured using F_{IS}, calculated according to Weir & Cockerham (1984) and tested for significance by 10,000 permutations in FSTAT 2.9.3. (Goudet, 1995). Mean pairwise relatedness within samples was calculated using the relatedness estimator, r_{qg}, of Queller & Goodnight (1989) in GENALEX with associated 95% confidence intervals

132 determined by 1000 bootstraps. Permutation of genotypes among all samples (999 times) was
133 used to calculate the upper and lower 95% confidence intervals for the expected range of r_{qg}
134 under a panmictic model.

135 Genetic differentiation was quantified by global and pairwise F_{ST} values, with
136 associated significance evaluated by 10,000 permutations (Goudet et al. 1996), using FSTAT.
137 Hierarchical analysis of molecular variance (AMOVA; Excoffier et al. 1992) was performed
138 in ARLEQUIN to partition genetic variance among groups of samples (F_{CT}) and among
139 samples within groups (F_{SC}) with significance levels of F_{CT} and F_{SC} tested using 1000
140 permutations. To help visualise F_{ST} results principal coordinates analysis (PCoA) was
141 performed on pairwise matrices. Mantel tests, as implemented in the IBDWS software
142 (Jensen et al. 2005) were used to test for correlation between pairwise linearised F_{ST} [F_{ST} /(1-
143 F_{ST})] (Rousset 1997) and shortest sea distances between sample sites (i.e. isolation by
144 distance - IBD). IBD tests were based on 10,000 randomisations and performed on
145 combinations of untransformed and log transformed genetic and geographical distances for
146 various pooled and partitioned arrangements of temporal, male and female samples.
147 Differentiation between samples was tested with global and pairwise exact *G*-tests in
148 GENEPOP (10,000 batches, 5000 iterations). The simulation method implemented in
149 POWSIM (Ryman & Palm 2006) was used to estimate the sample size dependent Type I and
150 Type II error probabilities of the exact *G*-tests. Genetic structuring was also investigated
151 using the Bayesian clustering method in STRUCTURE (Pritchard et al. 2000) both with and
152 without prior population information and with multiple parameter sets (i.e. with and without
153 admixture, and with and without correlated allele frequencies). Randomisation procedures in
154 FSTAT were used to detect significant differences in heterozygosity, A_R, F_{IS}, F_{ST} and
155 relatedness among user defined groups of samples following 10,000 permutations.

156 The assumption of selective neutrality of the microsatellite loci was assessed using the
157 FDIST outlier identification test (Beaumont & Nichols 1996) implemented in LOSITAN
158 (Antao et al. 2008) performed (i) globally (i.e. across groups of samples) and (ii) between
159 pairs of samples. Simulations were run for 10,000 replications, 95% confidence intervals
160 estimated using the options for neutral and forced mean F_{ST}. No differences were detected
161 between analyses assuming infinite allele model (IAM) and stepwise mutation models and so
162 only IAM results are presented.

163

164 ## RESULTS

165 ### Intrasample genetic variability and data power

166 A total of 2,777 individuals were assayed (mean sample size = 81.7), with an average
167 of 17.4 alleles detected per locus (range 5-34). Loci Cpag4 and Cpag5d8 had considerably
168 more alleles (n = 34 in both cases) than the other loci, with the next highest allele number
169 reported for Cpag1b9 (19 alleles). Each locus was polymorphic in all samples with very
170 similar levels of variation across all samples. No significant linkage disequilibrium between
171 loci was detected, either across all samples (data pooled) or in any single sample. Single
172 locus tests for conformity to Hardy Weinberg equilibrium (HWE) expectations for each of
173 the initial 34 samples (Table 1) revealed the largest number of significant deviations (at
174 critical P value = 0.05) for Cpag4 and Cpag3A2 which exhibited 19 and 12 significant test
175 results, respectively. No other locus exhibited more than 5 deviations out of 34 tests (at
176 critical P = 0.05). Multilocus tests of HWE and associated F_{IS} values were non-significant in
177 most samples (Table 1). All single- and multi-locus deviations from HWE were due to
178 heterozygote deficits. Application of MICRO-CHECKER (Van Oosterhout et al. 2006)
179 algorithms to adjust for potential null alleles in cases of single locus heterozygote deficits

resulted in no change to the magnitude and pattern of genetic differentiation revealed in subsequent tests and so results for unedited data are reported. Mean intra sample relatedness conformed to predictions of a panmictic model for all but two samples (Table 1).

POWSIM analysis indicated both considerable statistical power for *G* tests to detect population structure and low type I error rates, for various sample size permutations relative to this study (Table 2).

Detection of divergent selection effects

Significant differentiation was detected between males and females collected in the Gulmarsfjord (F_{ST} = 0.027, P < 0.0001; exact G test P < 0.0001). This differentiation was driven by a single locus (Cpag6c4b) which yielded a pairwise F_{ST} of 0.160 (P = 0.0001; Exact *G* test P = < 0.0001). Differentiation between the sexes was not significant upon exclusion of this locus (F_{ST} = 0.0048; P = 0.20; Exact *G* test P = 0.06)). Genotype proportions at Cpag6c4b conformed to HWE among both Gulmarsfjord males (P = 0.5) and females (P = 0.8), with both groups exhibiting nearly identical levels of variability at this locus, as well as at other loci. The locus-specific differentiation between the sexes was effected by a clear shift in respective allele frequency distributions (Fig. 2). The simulation based test for signals of selection within male and female samples identified Cpag6c4b as a positive outlier (Supplementary Fig 1; simulated F_{ST} smaller than Cpag6C4B F_{ST}, P = 0.9979) likely to be influenced by divergent selection.

Comparison of the Gulmarsfjord samples with all other samples revealed: (i) the distinctiveness of the Gulmarsfjord males; and (ii) relative similarity of the Gulmarsfjord females to all other samples (Table 3). The pronounced differentiation of the Gulmarsfjord

males from all other samples was also driven by locus Cpag6c4b (mean pairwise F_{ST} for Cpag6c4b = 0.169 (SD = 0.013); mean pairwise F_{ST} excluding Cpag6c4b = 0.003 (SD=0.003)). Outlier identification tests identified Cpag6c4b as a positive outlier in all pairwise and global tests which included the Gulmarsfjord males (Supplementary Fig. 2). Cpag6c4b allele frequency distributions among Gulmarsfjord females were similar to other samples (Fig 2) and pairwise comparisons including this sample yielded a mean pairwise F_{ST} - Cpag6c4b of 0.008 (SD = 0.013), which was similar to values based on the other seven loci (mean pairwise F_{ST} – excluding Cpag6c4b = 0.002 (SD = 0.004)). All outlier tests (pairwise and global) excluding the Gulmarsfjord males reported no significant outliers (Supplementary Fig. 3). Collectively these results indicate that, among the analysed samples, potential divergent selection effects at locus Cpag6c4b were only detectable in comparisons involving the Gulmarsfjord males.

Neutral Genetic structuring

Spatial / Temporal homogeneity among North Sea samples

Among the North Sea samples, excluding the Gulmarsfjord males, all intra-sample pairwise tests of differentiation between sexes were non-significant. Additionally, for those sites with temporally replicated samples, all intra-site comparisons were non-significant regardless of the arrangement of samples (i.e. whether tests were performed on samples pooled or segregated according to sex / time). All pairwise tests between sites yielded non-significant results, regardless of intra-site pooling / partitioning strategies. Upon pooling samples according to site all pairwise tests were non-significant (Table 3), as was global F_{ST} (F_{ST} = 0 .001; P = 0.057). The corresponding multilocus global G test was significant (P =

0.02), however this was due to a significant value at only one locus (Cpag3d7, P = 0.025), omission of which resulted in a non-significant global G (P = 0.066).

Genetic structuring within English Channel and Celtic / Irish Sea samples

Among the English Channel samples no significant pairwise differentiation was detected between sexes in any samples. Significant temporal differentiation was reported between the Hastings 2000 and 2006 samples (Table 3), with this temporal differentiation also evident in pairwise comparisons between the relevant sex-segregated samples (Hast-00 females vs. Hast-06 males F_{ST} = 0.0055 , P = 0.045, Exact G P = 0.038; Hast-00 females vs Hast-06 females F_{ST} = 0.007, P = 0.031, Exact G P < 0.01). Significant differentiation between temporal replicates was reported for both within-bay samples from this region (Newlyn and Brittany), as well as for Jersey. Pairwise differentiation between temporal replicate samples within sites in many cases exceeded that between sites and contributed to an overall pattern of low but significant global structuring within the English Channel (global F_{ST} = 0.004, P = 0.001; global G P < 0.0001) which did not show any consistent geographical or temporal pattern (see Table 3). Similar numerically small, yet significant, genetic structuring was also reported among Celtic / Irish Sea samples (global F_{ST} = 0.004, P = 0.001; global G P < 0.0001), driven by the differentiation of, and among, the more southern samples in the region (Table 3).

Inter-regional genetic structure

The two samples from the west of Ireland (NWIre and GalBay) were not significantly differentiated from each other but exhibited a high proportion of significant pairwise test

249 results against samples from other regions (Table 3). This differentiation was apparent in the
250 PCoA (Fig.3), which also highlighted the differentiation of the Saint Ives Bay sample (Celtic
251 Sea) with 27 out of 31 significant pairwise tests (Table 3). Examining pairwise test results
252 revealed that differentiation between samples was consistently small but in many cases
253 significant, and did not follow a coherent spatial / temporal pattern but rather similar to the
254 spatial / temporal patchiness reported in the English Channel and Celtic / Irish Seas. This
255 patchiness was not apparent in the STRUCTURE analysis which reported unanimous support
256 for a model of $K = 1$ in all analyses excluding the Gulmarsfjord males. Concordant with the
257 lack of spatial / temporal patterning revealed by pairwise tests no significant IBD was
258 detected (all test results $P > 0.1$) and AMOVA reported greater variation among samples
259 within regions than between regions (Table 4). AMOVA revealed similar patterns among
260 partitioned males and females, and randomisation tests indicated no significant differences
261 between the sexes for a number of indices (Table 5).

262

263 **DISCUSSION**

264 The present study represents the most geographically extensive investigation of brown
265 crab population genetic structure to date. The research employed fine- and regional-scale
266 spatial / temporal sampling, along with combined gene flow and kinship based analyses and
267 marker neutrality tests to elucidate the mechanistic underpinnings and eco-evolutionary
268 significance of patterns of genetic diversity (following recommendations by Waples 1998,
269 Nielsen et al. 2009, Iacchei et al. 2013). A striking feature of the results was the
270 differentiation of the Gulmarsfjord males from all other samples, including females collected
271 at the same site. This differentiation was driven by a single locus (Cpag6c4b) which was
272 found to have a significantly higher F_{ST} than expected under neutrality in all pairwise

comparisons involving this sample, suggesting divergent selection effects. Excluding the Gulmarsfjord males, all loci (including Cpag6c4b) conformed to neutral expectations and revealed numerically small but statistically significant differentiation among samples across the NE Atlantic. This global genetic structuring did not fit to an IBD model or an obvious hierarchical geographic pattern. Pairwise tests of differentiation (F_{ST} and exact *G*) revealed that the majority of comparisons were non-significant, including comparisons between geographically distant sites, but that a substantial number of comparisons exhibited significant differentiation which conformed to a model of chaotic genetic patchiness (CGP) in the sense that temporal and / or fine scale differentiation often exceeded that at larger spatial scales (Johnson & Black 1984, Hedgecock 1994, Selkoe et al 2006, Banks et al. 2007). Particular samples (discussed below) were associated with a high proportion of significant pairwise tests indicating geographically local or sample-specific effects. Overall, patterns of neutral genetic variation in brown crab indicate local and unstructured genetic differentiation occurring against a background of high gene flow throughout the studied region.

The positive outlier status of locus Cpag6c4b in all comparisons involving the Gulmarsfjord males suggests that this locus, or a linked genomic region, is subject to divergent selection effects. This result adds to a number of studies reporting selection effects apparent at microsatellite loci that were at some stage assumed to be neutral (Larsson et al. 2007, Skarstein et al. 2007, Westgaard & Fevolden 2007, Nielsen et al. 2009, Gaggiotti et al. 2009, White et al. 2010). Excluding locus Cpag6c4b, the Gulmarsfjord male sample was not significantly differentiated from the Gulmarsfjord female sample, or from most other samples. There are a number of potential explanations for such a pattern of locus-specific genetic differentiation. For example, the pattern could be generated without reproductive isolation through selection on individuals during early life stages followed by random mating each generation (i.e. differential genotype selection within a panmictic gene pool). At the

other end of the spectrum the pattern may reflect temporally stable reproductive isolation that is not detectable at neutral loci that lack the statistical power and / or are not at migration-drift equilibrium (Nielsen et al. 2009). Morphological, biochemical and genetic studies have demonstrated population differences among fjords, and between fjords and coastal areas, for a number of taxa (e.g. Jorstad & Naevdal 1989, Suneetha & Naevdal 2001, Oresland & Andre 2008, Teacher et al., 2013). While the allele frequency differences between brown crab sexes within the Gulmarsfjord may indicate gender-specific selection, mechanical mixing of individuals from differently adapted populations could also explain the differences. Evidence of female reproductive migration and lack of return migrations (Ungfors et al. 2007) suggests that the sampled Gulmarsfjord females may be allochthonous, while the male sample is composed of local (at least post settlement) individuals. The differentiation between the sexes may therefore reflect allele frequency differences, and by extension adaptive differences, in their respective parental populations and not necessarily differential selection between sexes *per se*. In this sense the pattern culd be considered similar to the mechanical mixing of differently adapted migratory north-east Arctic cod (NEAC) and sedentary Norwegian coastal cod (NCC) populations within fjords (Sarvas & Fevolden 2005, Fevolden et al. 2012). The Gulmarsfjord crab data are consistent with other studies indicating that features of fjords may drive local adaptation (Dick et al. 2014) with both salinity and depth highlighted as candidate features by Fevolden et al. (2012), and identified as drivers of selection in other systems (salinity – Nielsen et al 2009; depth – White et al. 2010). Future analysis of larval recruits would provide a means to investigate the relative roles of pre- and / or post settlement selection and dispersal in shaping the observed pattern while identification of underlying functional genetic differences and detection of other samples with similar adaptive fingerprints may help elucidate the environmental drivers.

322 The seemingly paradoxical pattern of CGP within broad-scale genetic homogeneity
323 reported here has also been documented in a variety of marine species (limpets - Johnson &
324 Black 1984; fish - Planes & Lenfant 2002, Selkoe et al. 2006; barnacles - Veliz et al. 2006).
325 Cautious interpretation of such patterns is recommended as when differentiation is low
326 multiple sources of artificial variance such as unrepresentative sampling (e.g. family / kin
327 sampling - Hansen et al. 1997, Waples 1998, Waples & Gaggiotti 2006) and statistical noise
328 (Waples 1998, Hedrick 1999, 2005) can be important and lead to false conclusions. Kin
329 aggregation is generally assumed to be a transient phenomenon limited to newly settled
330 recruits with little detectable signal in adult populations (Flowers et al. 2002, Planes et al,
331 2002, Selkoe et al. 2006; but see Iacchei et al. 2013), and as the analysed samples consisted
332 of mixed cohorts of adults kin aggregation would be an unlikely source of error. Furthermore,
333 mean kinship values provided no strong evidence of large proportions of closely related
334 individuals within samples. POWSIM analysis also indicated that the sample sizes conferred
335 low probability of Type I errors. Therefore, while the genetic differentiation only amounts to
336 slight differences in allele frequencies that may not have substantial evolutionary effects
337 (Waples 1998), they nonetheless signal changes in the composition that may be a useful tool
338 for better understanding recruitment dynamics and connectivity in this species (Selkoe et al.
339 2006, Knutsen et al. 2011).

340 Fine scale genetic patchiness against a background of high gene flow has been
341 variously attributed to three phenomena that may act in concert: large variances in individual
342 reproductive success (sweepstakes recruitment), limited mixing of larvae from genetically
343 different sources (larval cohesion) and local selection (Larson & Julian 1999). Sweepstakes
344 recruitment has been reported for a number of highly fecund (Type III) marine taxa, such as
345 brown crab, and may generate temporal / spatial genetic differentiation despite gene flow
346 when recruitment is variable. Even in the absence of genetically isolated source populations,

347 as might be the case here, larval cohesion (Selkoe et al. 2006) may enhance (Waples 2002),
348 and be effectively indistinguishable from sweepstake effects (Turner et al. 2007). The
349 divergent selection effect suggested for Gulmarsfjord males highlights the potential for fine-
350 scale selection in brown crab. However, outlier tests excluding this sample showed no
351 evidence of selection effects for any other locus / sample combination. Furthermore, the
352 temporal differentiation at a number of sites supports more prominent roles for processes like
353 sweepstakes recruitment or larval cohesion as components of recruitment variability, rather
354 than consistent selection effects. For example, the genetic patterns reported for the Hastings
355 samples, the most eastern site sampled in the English Channel, are readily compatible with
356 the proposed relationship between recruitment variability and genetic patchiness. This area
357 was identified *a priori* as a potential hotspot of recruitment variability (Derek Eaton,
358 unpublished study on larval abundance and modelling). As the signatures of such processes
359 are predicted to be diminished by postlarval dispersal (Planes & Lenfant 2002), the genetic
360 patchiness observed here must be considered a conservative reflection of the extent of
361 recruitment heterogeneity.

362 The CGP in brown crab was unusual in exhibiting a geographic pattern. In contrast to
363 substantial numbers of significant differences among samples within the Irish / Celtic Seas
364 and the English Channel, excluding the Gulmarsfjord males there were no significant
365 differences among North Sea samples (see Table 3), which together with the spatial /
366 temporal genetic homogeneity among Scandinavian samples described by Ungfors et al.
367 (2009) indicates an absence, or lower level, of genetic patchiness in the North Sea compared
368 to other regions studied. While such structuring likely reflects complex interactions between
369 life history and environmental variables, the geographic pattern permits identification of
370 specific factors that may be involved. North Sea brown crabs are significantly smaller than
371 English Channel crabs (Pawson 1995), and as brown crab fecundity is linked to female size

372 (Edwards 1979; Ungfors 2007) lower fecundity of North Sea crab may reduce the potential
373 extent of reproductive skews compared to those in the English Channel. For example, Palero
374 et al. (2011) suggested that the selected harvesting of large females reduced variance in
375 reproductive success in *Panulirus elephas*. McKeown & Shaw (2008b) posited the genetic
376 monogamy of female brown crab as another life history feature that may increase variance in
377 reproductive success among individuals, but they only analysed samples from the English
378 Channel (which exhibited CGP). Multiple paternity has been reported in a number of closely
379 related species (e.g. Jensen & Bentzen 2012) and may occur in brown crab from other areas,
380 wherein it could serve to reduce variance in reproductive success among males. Seascape
381 factors may directly influence, or interact with the genetic signatures of, variance in
382 reproductive success (Banks et al. 2007). The English Channel exhibits a higher degree of
383 fine scale oceanographic complexity and coastal heterogeneity, in comparison to the North
384 Sea (no CGP), both factors that have been linked to localised sweepstakes recruitment in sea
385 urchins (Banks et al. 2007). Likewise, the high proportion of significant pairwise tests
386 reported for samples collected within semi-enclosed bays (Newlyn, Brittany, Galway, St.
387 Ives), suggests an association between genetic differentiation and habitat structure as an
388 additional component of fine-scale seascape structuring (Selkoe et al. 2010). Comparative
389 studies among taxa with common and contrasting life history strategies will be necessary to
390 elucidate the specific drivers of genetic variation (e.g. Selkoe et al. 2010); however, the
391 brown crab data highlight the fact that genetic structuring may be driven by factors other than
392 dispersal.

393 Pairwise tests reported a general pattern wherein the West of Ireland samples were
394 differentiated from all samples except those collected in the northern North Sea. This may
395 reflect geographically coherent connectivity. Sotelo et al. (2008) reported spider crab from
396 the west of Ireland to be genetically distinct from more southern samples. However, in

general the genetic patterns for brown crab cannot readily be interpreted in the context of population connectivity/isolation. While the described factors driving CPG may lead to an underestimation of migration rate (*m*), changes in dispersal behaviour between different life stages may result in broad scale genetic homogeneity masking of spatial gene flow restrictions (e.g. Berry et al. 2012), Partitioned analysis of sexes provided no evidence of greater structuring among male crabs that might be indicative of temporally stable spatial patterns of larval self-recruitment. Furthermore, the difficulties of deriving quantitative estimates of gene flow and dispersal from subtle genetic structure among large populations (Whitlock & McCauley 1999; Palsboll et al. 2007; Hellberg 2009), and discrepancy between levels of gene flow needed to limit genetic differentiation and dispersal needed to replenish stocks (Hauser & Carvalho 2008) are fundamental issues. Therefore, while the low level of genetic structure throughout the studied region is compatible with high gene flow it cannot be ruled out that there is significant isolation of stocks on timescales of interest to management. Resolution of such spatial stock structure may be beyond the level of neutral genetic markers and benefit from complementary analysis of markers under selection (Canino et al. 2005).

This study has implications for sustainable management of the brown crab fishery. The detection of adaptive diversification should enhance appreciation of local adaptation as a component of species biodiversity, and highlights a potential danger of indiscriminate harvesting of differentially adapted units on local scales. Stochastic recruitment variability suggested to underpin genetic patchiness may decrease resilience of local stocks to fishing and increase unpredictability in recovery (Kuparinen et al 2014), and will necessitate a tailoring of the spatial scale of management (spatial bet hedging) according to biological and physical drivers of such recruitment variability. This study provides a baseline for future genetic studies of brown crab, needed to understand recent events such as expansions in census population size within the English channel (Molfese et al. 2014), and further

demonstrates how intraspecific biodiversity and population viability is influenced by complex species-environment interactions other than dispersal.

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Table 1. Brown crab sample information, including geographical region (used in AMOVA), date of collection and sample composition (i.e. numbers of males/females where identified at time of sampling; * denotes the inclusion of sample in sex-segregated analysis). Multilocus genetic variability measures: N_A (allele number); A_R (allele richness); H_O (observed heterozygosity); H_E (expected heterozygosity); F_{IS} (standardised genetic variance within samples - * denotes significant deviations from HWE expectations); within-sample relatedness (r_{qg} - * denotes values significantly different from expectations of a panmictic model).

Region	Sample site	Sample code	Sample number	Collected	Sample Composition			Total	Mean N_a/locus	Mean A_R	H_O	H_E	F_{IS}	r_{qg}
					Male	Female	Gender unknown							
West of Ireland	Northwest Ireland	NWIre	1	Jul-07			30	30	8.00	7.867	0.601	0.6571	0.086*	-0.013
West of Ireland	Galway Bay	GalBay	2	Jul-07			46	46	8.63	7.567	0.589	0.6456	0.089*	0.029
Irish/Celtic Sea	Southeast Ireland	SEIre	3	Jul-07			31	31	8.38	8.176	0.624	0.646	0.034	0.011
Irish/Celtic Sea	Aberystwyth	Aber	4	Aug-00	8	61*		69	10.00	8.221	0.622	0.630	0.014	0.058*
Irish/Celtic Sea	Newquay	Newq	5	Jun-06	43*	51*		94	10.88	8.196	0.658	0.645	-0.02	0.010
Irish/Celtic Sea	St. Ives Bay	SI Bay	6	Sep-07			55	77	11.13	8.242	0.676	0.649	-0.042	0.008
Irish/Celtic Sea	Pendeen	Pen	7	Jun-06	51*	51*		102	10.75	7.790	0.598	0.652	0.083*	0.004
English Channel	Newlyn Bay	NewBay	8	Sep-00	1	83*		84	11.13	8.357	0.689	0.673	-0.024	-0.019
English Channel	Newlyn Bay	NewBay	8	Oct-07			81	81	10.50	7.983	0.638	0.664	0.041*	0.001
English Channel	Brittany Bay	Brit Bay	9	Sep-00			58	58	9.00	7.696	0.651	0.660	0.014	0.009
English Channel	Brittany Bay	Brit Bay	9	Jul-06		102*		102	11.38	8.366	0.621	0.646	0.042*	0.026
English Channel	Brittany-Offshore	Brit offshore	10	Oct-06	56*	58*		114	11.50	8.221	0.626	0.660	0.053*	-0.003
English Channel	Jersey	Jer	11	Sep-00	40*	32*		72	10.13	7.918	0.662	0.653	-0.014	0.020
English Channel	Jersey	Jer	11	Sep-07			84	84	10.88	8.230	0.653	0.642	-0.018	0.037*
English Channel	Guernsey	Guer	12	Sep-07			80	80	10.50	8.164	0.659	0.659	0	0.000
English Channel	PortScatho	Portscat	13	Jun-06			136	136	11.63	7.915	0.675	0.640	-0.055	0.028
English Channel	Plymouth	Ply	14	Oct-00	9	52*	2	63	10.25	8.129	0.645	0.633	-0.018	0.040
English Channel	Start Point	StartP	15	Jul-06	62*	71*		133	11.75	8.084	0.643	0.649	0.009	0.007
English Channel	Lyme Bay	Lyme	16	Jul-07			52	52	9.88	8.504	0.656	0.645	-0.016	0.027

Table 1. Continued

Region	Sample site	Sample code	Sample number	Collected	Sample Composition				Mean N_a/locus	Mean A_R	H_O	H_E	F_{IS}	r_{qg}
					Male	Female	Gender unknown	Total						
English Channel	Swanage	Swan	17	Jun-06	44*	11		55	9.50	7.97	0.645	0.672	0.041	-0.016
English Channel	Brighton	Brighton	18	Sep-07			65	65	9.63	7.78	0.625	0.648	0.035	0.022
English Channel	Hastings	Hast	19	Aug-00	5	67*		72	10.25	8.23	0.601	0.665	0.097*	-0.015
English Channel	Hastings	Hast	19	Oct-06	54*	108*		162	12.25	8.32	0.628	0.645	0.026	0.019
North Sea	Harwich	Har	20	Jun-00	47*	15	1	63	9.88	8.20	0.688	0.665	-0.033	-0.014
North Sea	Harwich	Har	20	May-05	101*	58*		159	13.13	8.47	0.644	0.656	0.019	0.001
North Sea	Norfolk	Norf	21	Jun-00	39*	39*	2	80	11.13	8.23	0.641	0.652	0.017	0.014
North Sea	Bridlington	Brid	22	Aug-01	44*	40*		84	10.75	8.22	0.629	0.652	0.036	0.010
North Sea	Bridlington	Brid	22	Jun-06	50*	56*		106	11.63	8.15	0.628	0.652	0.036	0.010
North Sea	Northumberland	North	23	Jun-00	48*	46*		94	11.25	8.39	0.659	0.653	-0.011	0.004
North Sea	Northumberland	North	23	Sep-05	56*	9		65	10.50	8.34	0.612	0.647	0.054*	0.014
North Sea	Orkney-Hoy	Ork-Hoy	24	Jun-02	43*	40*	15	98	11.25	8.26	0.649	0.643	-0.008	0.020
North Sea	Orkney-Sanday	Ork-Sand	25	Jun-02			38	38	9.25	8.58	0.686	0.682	-0.006	-0.041
North Sea	Shetland	Shet	26	Jun-07				48	9.63	8.09	0.672	0.665	-0.010	-0.017
North Sea	Gulmarsfjord	Gulm	27	Jun-02	41**	39*		80	10.00	7.82	0.627	0.651	0.037	0.014

Table 2. Estimated statistical power for detecting various true levels of population differentiation (F_{ST}) by means of Fisher's exact *G* tests in pairwise comparisons involving various permutations of sample sizes relative to this study (n = 30: minimum sample size used in pairwise tests; n = 84: average sample size; n =120: representative of larger sample sizes employed). Power is expressed as the proportion of simulations reporting statistical significance at the 0.05 level. Bold values denote values obtained for simulated F_{ST} = 0 (Type I error), non-bold denote values obtained for simulated F_{ST} = 0.0025.

	sample n= 30	sample n= 84	sample n= 120
sample n= 30	**0.049**/0.409		
sample n= 84	**0.065**/0.699	**0.047**/0.981	
sample n= 120	**0.055**/0.784	**0.048**/0.997	**0.062**/1

Table 3. Pairwise F_{ST} values between all samples, with intraregional comparisons outlined and shaded. See Table 1 for sample codes. PF_{ST} and Exact *G* tests yielded similar patterns of significance and so only PF_{ST} are reported (italics = P < 0.05; underlined = P < 0.01; bold = P < 0.001).

	NWIre	GalBay	SEIre	Aber	Newq	SI Bay	Pen	NewBay-2000	NewBay-2007	Brit Bay -2000	Brit Bay-2006	Brit offshore	Jer-2000	Jer-2007
GalBay	0.003													
SEIre	0.003	**0.019**												
Aber	*0.007*	**0.018**	0.006											
Newq	0.004	**0.013**	0.005	-0.005										
SI Bay	0.004	**0.022**	**0.015**	*0.005*	0.005									
Pen	**0.013**	**0.024**	0.010	*0.005*	*0.004*	*0.004*								
NewBay-2000	0.010	**0.015**	0.011	0.007	*0.004*	**0.011**	0.002							
NewBay-2007	0.011	**0.017**	**0.021**	**0.013**	0.007	**0.012**	0.007	0.006						
Brit Bay -2000	**0.019**	**0.022**	**0.017**	0.008	0.007	**0.018**	0.008	0.009	**0.013**					
Brit Bay-2006	0.003	**0.014**	0.002	0.001	-0.001	0.003	0.002	0.005	0.008	**0.013**				
Brit offshore	*0.008*	**0.013**	0.011	*0.004*	0.001	0.005	0.002	0.001	0.002	0.002	*0.003*			
Jer-2000	0.013	**0.025**	0.007	0	0.002	0.008	0.001	0.001	**0.008**	0.004	0.002	0.001		
Jer-2007	*0.008*	0.009	0.012	0.007	0.002	0.008	*0.004*	0.006	*0.005*	0.009	0.003	0.002	0.008	
Guer	*0.008*	**0.011**	0.012	0.003	0.001	*0.006*	0.001	-0.001	*0.005*	0.009	0.001	0.001	0.002	0.003
Portscat	*0.006*	**0.012**	0.012	0.001	-0.002	*0.004*	*0.003*	0.002	0.003	**0.011**	-0.001	0.001	0.001	0.003
Ply	**0.023**	**0.019**	0.013	*0.007*	0.006	**0.019**	0.007	0.001	0.008	0.008	0.007	*0.004*	0.004	*0.006*
StartP	0.012	**0.018**	*0.009*	0.002	0.001	0.006	0	0.002	0.008	0.002	*0.004*	-0.001	0.001	*0.002*
Lyme	0.007	**0.020**	*0.011*	-0.001	0.003	0.004	*0.007*	*0.007*	0.014	**0.014**	0.002	*0.005*	0.003	**0.014**
Swan	0.007	*0.008*	*0.008*	**0.015**	*0.007*	0.011	0.002	*0.006*	*0.007*	0.009	*0.006*	0.002	0.009	0.002
Brighton	*0.009*	0.010	0.013	0.003	0.003	0.012	0.005	0.001	0.003	0.010	*0.004*	0.002	0.003	0.004
Hast-2000	0.017	0.017	0.016	**0.011**	**0.008**	**0.022**	**0.011**	0.003	**0.012**	0.003	**0.013**	*0.005*	0.007	0.007
Hast-2006	*0.006*	0.013	0.012	0.002	0	0.003	0	0.003	0.006	0.008	0	0.001	0.003	0.002
Har-pooled	*0.006*	**0.011**	*0.008*	0.002	0	**0.007**	0.002	0.001	**0.007**	0.007	*0.002*	0.001	0.002	0.004
Norf	**0.018**	**0.017**	0.011	*0.009*	0.008	**0.022**	**0.008**	0.002	**0.016**	*0.005*	**0.014**	0.006	0.003	**0.011**
Brid-pooled	0.011	0.009	0.014	*0.004*	0.005	**0.014**	**0.009**	*0.003*	**0.012**	0.007	0.007	*0.003*	0.005	0.008
North-pooled	0.011	**0.017**	0.009	0.003	0.002	**0.014**	0.002	0.001	**0.011**	*0.004*	0.005	0.001	0.001	0.005
Ork-Hoy	0.012	**0.015**	*0.008*	0.003	*0.004*	**0.012**	0.003	0.002	**0.012**	*0.005*	*0.003*	*0.003*	0.001	0.005
Ork-Sand	0.001	0.006	0.009	0.005	0	*0.006*	0.002	-0.002	-0.001	**0.013**	0	-0.001	0.004	0.003
Shet	0.007	0.013	*0.008*	0.004	-0.007	*0.007*	0.002	-0.001	0.003	0.003	0.003	-0.003	0.002	0.003
Gulm-females	0.003	0.004	0.005	0.004	0.002	0.012	0.005	-0.001	*0.008*	*0.010*	0.002	0.004	*0.007*	0.002
Gulm-males	**0.028**	**0.035**	**0.037**	**0.028**	**0.026**	**0.036**	**0.030**	**0.027**	**0.036**	**0.029**	**0.030**	**0.025**	**0.032**	**0.027**

Table 3 continued

	Guer	Portscat	Ply	StartP	Lyme	Swan	Brighton	Hast-2000	Hast-2006	Har-pooled	Norf	Brid-pooled	North-pooled
Guer													
Portscat	-0.001												
Ply	0.002	*0.004*											
StartP	0.001	*0.002*	*0.004*										
Lyme	0.003	0.001	0.011	*0.007*									
Swan	0.004	0.008	**0.012**	*0.004*	**0.016**								
Brighton	-0.001	-0.003	0	*0.004*	*0.007*	0.010							
Hast-2000	*0.004*	0.009	0.002	0.003	**0.015**	0.009	0.003						
Hast-2006	0	-0.005	0.005	0.001	0.003	0.005	0.003	0.009					
Har-pooled	-0.001	0.001	*0.004*	0.001	0.003	*0.004*	0.002	**0.007**	0				
Norf	0.003	0.008	0	0.005	0.009	**0.011**	*0.006*	0.004	0.008	0.004			
Brid-pooled	0.002	0.003	0.005	0.003	*0.005*	0.009	0.003	*0.004*	0.005	0.001	0.003		
North-pooled	0.001	*0.003*	0.003	0	*0.005*	0.005	0.003	0.005	0.001	-0.001	0.003	0	
Ork-Hoy	0.001	*0.003*	0.001	0.001	*0.006*	*0.005*	0.003	*0.005*	0.002	0	0.001	0.001	-0.002
Ork-Sand	-0.002	-0.002	0.005	0.002	0.004	0.001	-0.002	0.005	-0.001	-0.001	0.004	0.002	0.001
Shet	-0.002	0	0.001	-0.001	0.005	0.004	-0.002	-0.002	0.002	-0.001	0.004	0	0
Gulm-females	-0.001	0.001	0.001	0.002	*0.009*	0.004	-0.004	0	0.002	0	0.005	0.001	0.002
Gulm-males	**0.024**	**0.028**	**0.035**	**0.025**	**0.032**	**0.032**	**0.025**	**0.029**	**0.026**	**0.027**	**0.030**	**0.026**	**0.026**

730 **Table 3.** Continued

	Ork-Hoy	Ork-Sand	Shet	Gulm-females
Ork-Hoy				
Ork-Sand	0.003			
Shet	0.002	-0.001		
Gulm-females	0.001	-0.005	-0.003	
Gulm-males	**0.026**	**0.027**	**0.024**	**0.027**

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734 **Table 4.** Analysis of molecular variance (AMOVA) across the three main sampling regions
735 (Celtic/Irish Sea; English Channel; North Sea), using either all data or sex segregated data
736 (see Table 1), and temporal samples within sites pooled or unpooled. F_{CT} = genetic variance
737 among regions; F_{SC} = genetic variance among samples within regions.

		F_{CT}	F_{SC}
All individuals	Temporal samples unpooled	0.001 ($P < 0.001$)	0.003 ($P < 0.001$)
	Temporal samples pooled	0.001 ($P < 0.001$)	0.002 ($P < 0.001$)
Males only	Temporal samples unpooled	0.002 ($P = 0.010$)	0.002 ($P = 0.040$)
	Temporal samples pooled	0.002 ($P < 0.001$)	0.002 ($P = 0.019$)
Females only	Temporal samples unpooled	0.001 ($P = 0.059$)	0.003 ($P <0.001$)
	Temporal samples pooled	0.001 ($P = 0.018$)	0.0018 ($P = 0.012$)

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739 **Table 5.** Comparative analysis of genetic diversity indices for brown crab females and males,
740 and corresponding *P* values from 2-tailed tests.

	Female	Male	Two tailed *P*
A_R	7.709	7.755	0.674
H_o	0.643	0.632	0.330
F_{IS}	0.012	0.032	0.186
F_{ST}	0.003	0.003	0.638
R_{qg}	0.007	0.005	0.611

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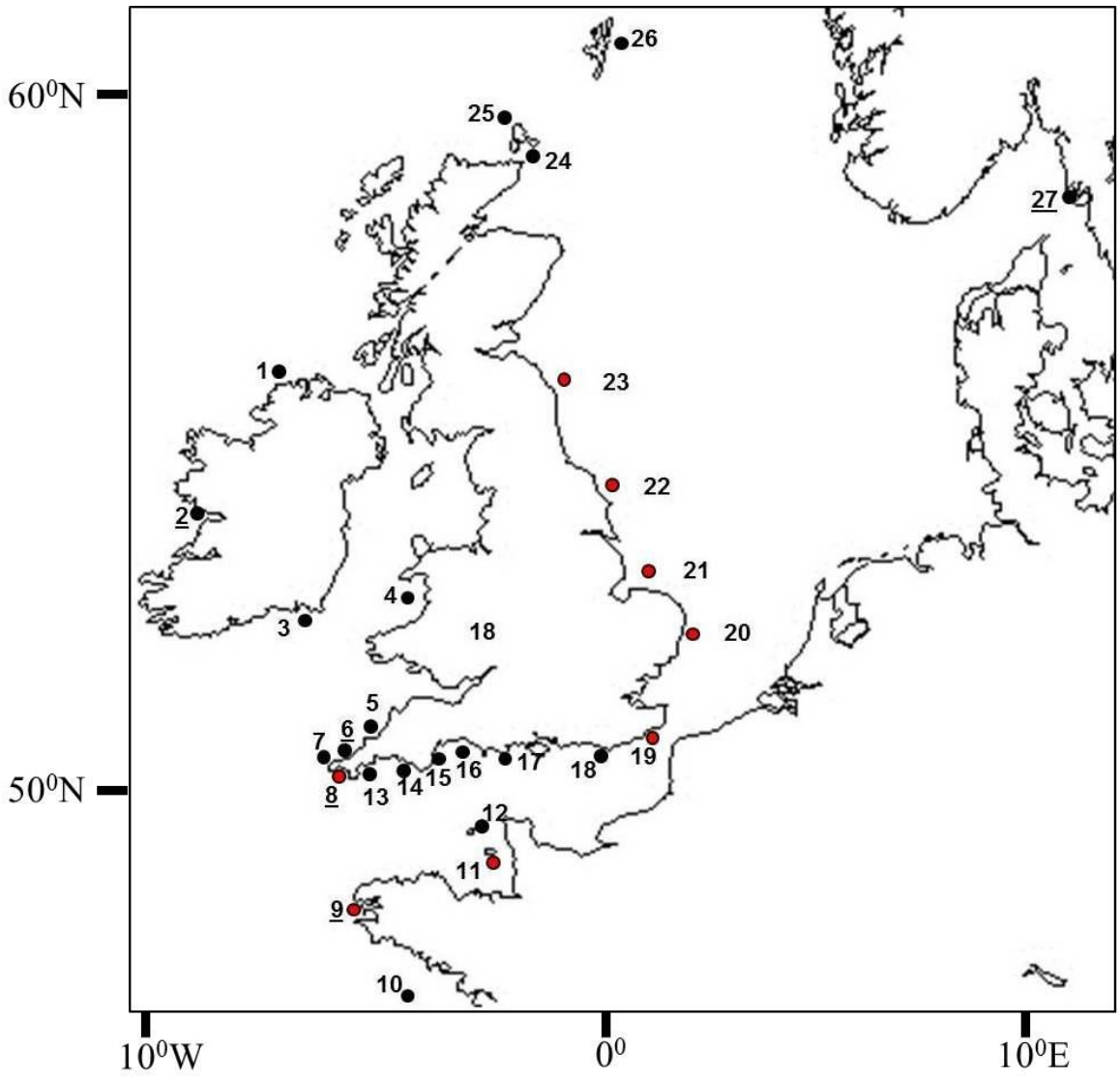

Figure 1. Brown crab sample sites (see Table 1 for site details). Red discs denote sites with temporal replicates, while underlined numbers highlight sample sites within semi-enclosed bays and fjords.

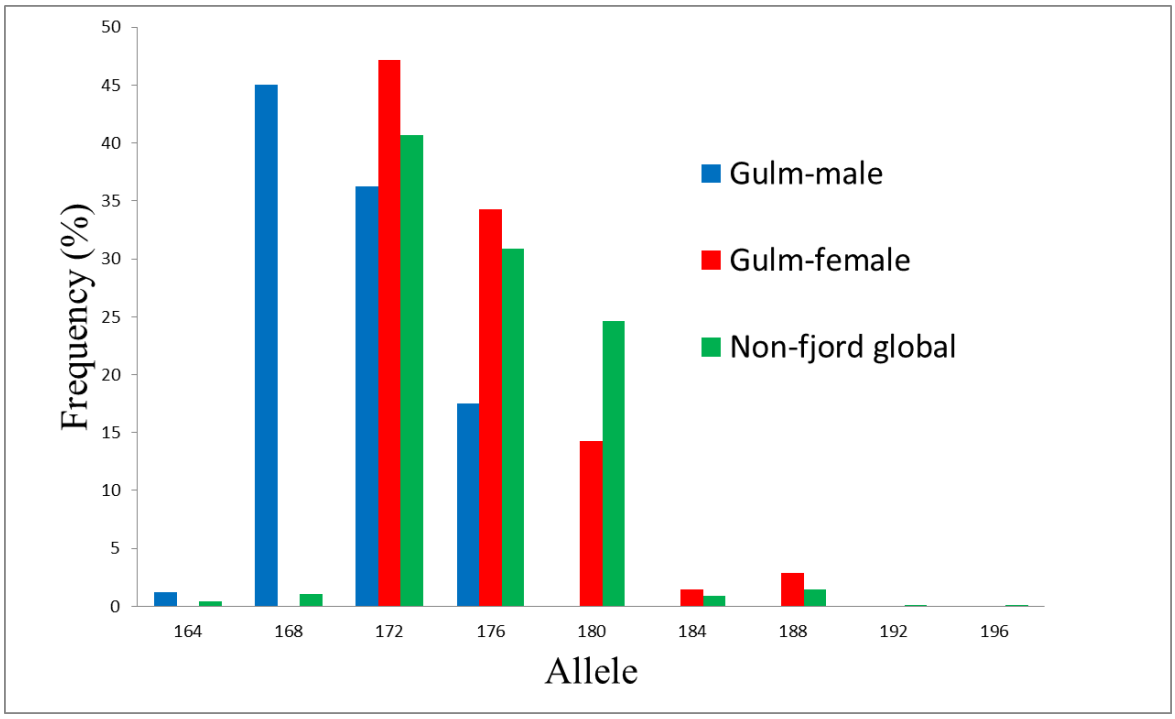

Figure 2. Allele frequencies at microsatellite locus Cpag6c4B for Gulmarsfjord males, Gulmarsfjord females, and for all other 'non-fjord' samples pooled.

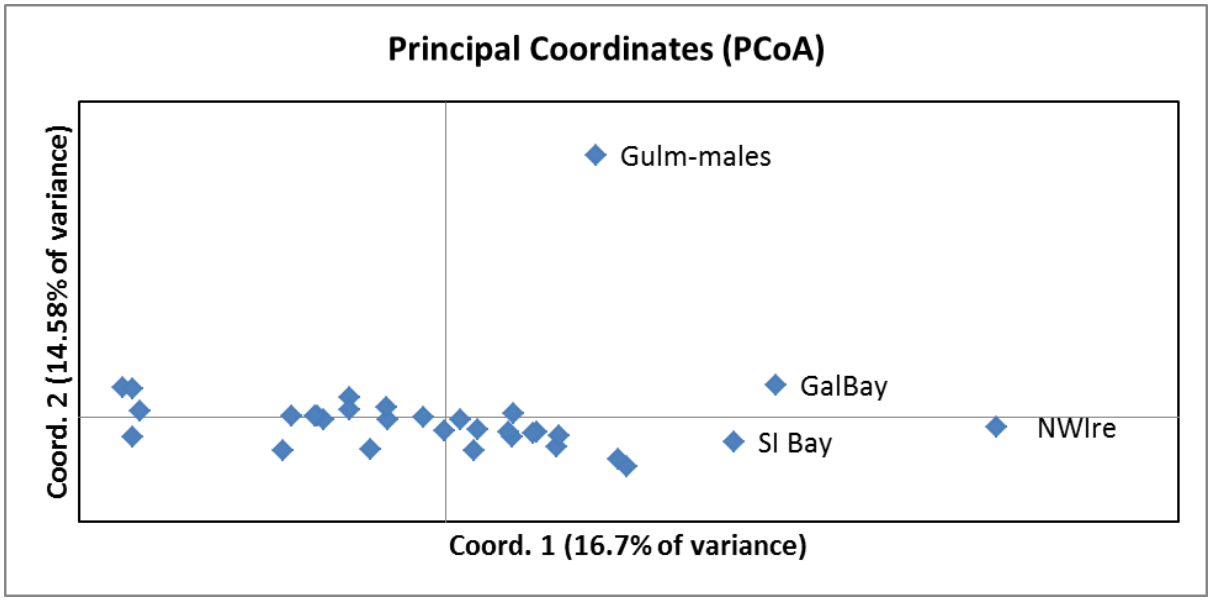

Figure 3. Principal coordinates analysis of multi-locus pairwise F_{ST} .